

PATENT APPLICATION

Method and Apparatus for Inspecting DNA and Method for Detecting Fluorescence

Inventors: **Yoshitada Oshida**
Residence: Chigasaki, Japan
Citizenship: Japan

Satoshi Takahashi
Residence: Hitachinaka, Japan
Citizenship: Japan

Taisaku Seino
Residence: Tsuchiura, Japan
Citizenship: Japan

Kenji Yasuda
Residence: Tokyo, Japan
Citizenship: Japan

Assignee: **Hitachi, Ltd.**
6, Kanda Surugadai 4-chome
Chiyoda-ku, Tokyo, Japan
Incorporation: Japan

Entity: Large

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

20053976.01302

- 1 -

METHOD AND APPARATUS FOR INSPECTING DNA AND METHOD
FOR DETECTING FLUORESCENCE

BACKGROUND OF THE INVENTION

The present invention relates to a method and an apparatus for irradiating DNA marked with fluorescence or a fluorescent material with excitation light to inspect the DNA and a fluorescence detection method and more particularly to a method and an apparatus for detecting and inspecting DNA marked with a plurality of kinds of fluorescence samples or a plurality of kinds of fluorescent materials at high speed.

As a method for irradiating fluorescence-marked DNA with excitation light to inspect the DNA, there is a method for focusing laser light constituting excitation light on a sample as a single spot beam to detect fluorescence and collecting a fluorescence image by means of scanning of the excitation light and the sample. In detection of a plurality of fluorescent marks, a desired all area of a sample is first scanned with first excitation light to obtain a fluorescent image of a first fluorescent mark and the desired all area of the sample is then scanned with second excitation light again to collect the fluorescent image of a second fluorescent mark.

10053975-011802

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a high-speed detectable DNA inspection method and apparatus.

5 In the conventional method, a desired area is scanned with excitation light having different wavelength in accordance with a plurality of fluorescent marks and this scanning is repeated by the number of marks. In order to change a relative position of a
10 single spot beam and a sample to inspect a desired area at high speed, it is necessary to move a stage on which the sample is placed at high speed. However, when the stage is moved at high speed, a considerable time is required for acceleration and deceleration for driving
15 the stage since reciprocating motion is needed.

Further, when the scanning using the stage is repeatedly made for each wavelength of the excitation light, a positional shift or deviation occurs in a combined image of fluorescent images obtained by means
20 of excitation lights when a driving accuracy of the stage and a reproduction accuracy are not sufficient. Consequently, high-speed and high-accuracy inspection of DNA required increasingly in future cannot be attained.

25 In order to solve the above problems, the present invention comprises measures described below.

A sample having a DNA piece added with a plurality of L kinds of fluorescence-marked materials

1.0053976.011802

combined to corresponding DNA is irradiated with a plurality of M kinds of minute spot excitation lights in accordance with the fluorescence-marked materials. Fluorescence intensities obtained in accordance with

5 the fluorescence-marked materials are separately detected by means of the plurality of M kinds of minute spot excitation lights. The separately detecting operation of the plurality of fluorescent marks is made by changing a position on the sample irradiated with

10 the spot excitation lights over a desired area by the number of times smaller than the number L of kinds of the fluorescence-marked materials, more preferably once, in order to detect the fluorescence intensities, to thereby inspect the DNA added with the plurality of

15 kinds of fluorescence-marked materials.

Further, when the plurality of kinds of minute spot excitation lights are a plurality of minute multi-spot excitation lights, respectively, a plurality of points about the fluorescent marks can be detected

20 at the same time to attain high-speed detection.

When different positions on the sample from one another are irradiated with the plurality of kinds of multi-spot excitation lights, fluorescence components from the fluorescent marks excited by the

25 plurality of kinds of excitation lights can be separately detected with high accuracy without causing fluorescence components to impede one another as noise.

Irradiation with the plurality of kinds of

10053976-011802

multi-spot excitation lights is made at the same time and fluorescence obtained by the plurality of kinds of minute spot excitation lights in accordance with the fluorescence-marked materials is detected by a

5 plurality of weak-light detection elements in accordance with respective excitation lights. The fluorescence intensities obtained in accordance with the fluorescence-marked materials are detected separately. Thus, in the photon-counting method used particularly
10 when weak fluorescence is detected, the photon-counting can be made at the same time with respect to respective fluorescence components to make inspection at high speed in wide dynamic range.

Irradiation with the plurality of kinds of
15 minute spot excitation lights is made in time series manner in accordance with wavelengths of the excitation lights and fluorescence obtained by the plurality of kinds of minute spot excitation lights in accordance with the fluorescence-marked materials is detected by a
20 common weak-light detection element to respective excitation lights. The fluorescence intensities obtained in accordance with the fluorescence-marked materials are detected separately. Thus, in detection using the multi-spot, particularly, the weak-light
25 detection element and its peripheral circuit become inexpensive.

Substantially the same position is irradiated with the plurality of kinds of minute spot excitation

10053976.011802

lights. Thus, detection by means of respective excitation lights can be made at the same place and detection with high accuracy can be made without any shift or deviation due to yawing and rolling of a stage in a
5 combined image.

The plurality of kinds of minute spot excitation lights are turned on and off in different time zone within a time that a relative position of the spot excitation lights and the sample to be irradiated is
10 changed by substantially one pixel. Thus, the respective fluorescent marks can be detected with high accuracy without mixing noise one another.

The plurality of kinds of minute spot excitation lights are changed stepwise at respective excitation light intensity levels within a time that a
15 relative position of the spot excitation light and the sample to be irradiated is changed by substantially one pixel to detect the fluorescence intensity at each step, so that detection is made over a wide dynamic
20 range at a high speed.

The DNA inspection apparatus according to the present invention is configured as follows. That is, the DNA inspection apparatus comprises one to a plurality of light sources for emitting lights having
25 wavelengths different from one another, a plurality-of-wavelength excitation optical system for irradiating a DNA sample added with a plurality of fluorescence-marked materials with lights having the plurality of

10053976-011802

wavelengths from the light sources as minute spot
excitation lights, a wavelength separation fluorescence
detection system for separately detecting fluorescence
intensities obtained by the respective excitation
5 lights in accordance with the fluorescence-marked
materials, a driving stage for changing a relative
position of the minute spot excitation lights and the
DNA sample over a desired area, and a processing unit
for driving the driving stage so that the relative
10 position of the minute spot excitation lights and the
DNA sample is changed over the desired area by the
number of times smaller than the number of kinds of the
fluorescence-marked materials so as to construct image
information of the plurality of fluorescence-marked
15 DNAs on the sample from fluorescence detection informa-
tion obtained by the detection system and the stage
position information obtained by scanning by the number
of times smaller than the number of kinds of the
fluorescence-marked materials over the desired area.

20 Further, the present invention is applied to
not only inspection of the fluorescence-marked DNA but
also general fluorescent material emitting fluorescence
peculiar to molecules such as protein.

According to the present invention, DNA to be
25 inspected including the plurality of fluorescent marks
or the samples containing the plurality of kinds of
fluorescence materials can be detected by scanning
within a desired detection spread by the number of

10053976-011802

times smaller than the number of kinds of the samples containing the fluorescence materials or fluorescent marks, more preferably once, to thereby make it possible to attain detection extraordinarily fast as compared with the prior art. Consequently, a large number of objects to be inspected can be detected and inspected at high speed to attain largely temporal and economical effects.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view illustrating the whole structure of a DNA inspection apparatus according to the present invention;

Fig. 2 is a plan view showing multi-spot excitation light within a detection field of vision according to the present invention;

Fig. 3 is an enlarged plan view of multi-spot excitation light according to the present invention;

Fig. 4 is a front view showing part of an optical system in which paths of excitation light and fluorescence according to the present invention are shown;

Fig. 5 is a front view showing an example of a fluorescence detection optical system according to the present invention;

Fig. 6 is a front view illustrating a photo-multiplier showing an example of fluorescence detection spots on the photo-multiplier according to the present

20053975.01802

invention;

Fig. 7 is a front view illustrating a photo-multiplier showing another example of fluorescence detection spots on the photo-multiplier according to
5 the present invention;

Fig. 8 is a diagram representing a relation of a plurality of fluorescence samples and the photo-multiplier according to the present invention;

Fig. 9 is a perspective view showing a
10 plurality-of-wavelength excitation optical system according to the present invention;

Fig. 10 is a graph showing a relation of temporal change in excitation light intensities and detection timings according to the present invention;

15 Fig. 11 is a graph showing a relation of temporal change in excitation light intensities and detection timings according to the present invention;

Fig. 12 is a graph showing a photon counting and detecting method according to the present invention;
20 tion; and

Fig. 13 is a perspective view showing a fluorescence detection unit according to the present invention.

DESCRIPTION OF THE EMBODIMENTS

25 Fig. 1 illustrates an embodiment according to the present invention. Numeral 1 denotes a control unit and numeral 5 denotes a sample board on which a

10053976 "011802
208110" 965500F

sample including fluorescent marks or fluorescence substance is placed. Numerals 21 and 22 denotes an excitation light source and a plurality-of-wavelength excitation optical system for irradiating the sample with minute spots of excitation light, respectively, described later with reference to Fig. 9. That is, the excitation light source 21 constitutes part of a light source composed of a plurality of semiconductor lasers having a wavelength of, for example, 635 nm and an excitation optical system for irradiating the sample with minute multiple spots of light emitted from the light source. The plurality-of-wavelength excitation system 22 constitutes part of a light source formed of a YAG laser having a wavelength of 532 nm and an excitation optical system for irradiating the sample with minute multiple spots of laser light emitted from the light source. The number of minute multiple spots is 64, 32, 16 or 8 and the number of minute multiple spots is selected therefrom in accordance with a specification of an inspection system. 64 laser beams having the wavelengths of 635 nm and 532 nm emitted from the excitation light source 21 and the plurality-of-wavelength excitation system 22, respectively, form 64 multi-spot spatial images having the respective wavelengths on positions 21A and 22A by means of beam alignment units 2101 and 2201, respectively. The multiple spot spatial images are focused on the sample put on the sample board 5 as multi-spot excitation

20250101 09:59:30

images indicated by 21B and 22B as shown in Fig. 2 by means of a tubular lens or a focusing lens 24 and an objective lens 3.

More particularly, as shown in Fig. 3, the M
5 (64) spots each having a diameter d are arranged at a pitch P_y on the sample. Numeral 21B represents the multi-spot having the wavelength of 635 nm and numeral 22B represents the multi-spot having the wavelength of 532 nm. The spaces P_x between the spots 21B and 22B
10 are not equal to one another and are several tens to several hundreds micrometers in the embodiment. The spaces can be made equal to one another depending on a specification to be targeted as described later.

Numerals 31 and 32 of Fig. 1 denote wave-
15 length selection beam splitters which transmit light beams having wavelengths of 635 and 532 nm of the excitation light, respectively. When fluorescence material on the sample is excited by the multiple spots, the fluorescence material absorbs the excitation
20 light having the wavelength of 635 nm (λ_1) impinging on positions 2101, 2102, ..., 21M of the excited minute spots and emits fluorescence having a wavelength λ_1' slightly longer than the above wavelength when fluorescence material ML1 emitting fluorescence is present.
25 The emitted fluorescence passes through the objective lens 3 having a large numerical aperture (NA) as shown in Fig. 4 and advances as shown by broken line with double arrows. The emitted fluorescence is then

20250926 011802

reflected by the wavelength selection beam splitter 32
and further reflected by wavelength selection beam
splitters 33 and 34 having the same wavelength
selection characteristic as the beam splitter 32 (or
5 reflected by a mirror 33' having a high reflectivity
and a wavelength selection beam splitter 34' which
transmits fluorescence λ_2' and reflects λ_1' as described
later) to enter a wavelength separation fluorescence
detection system 100 described later in detail with
10 reference to Figs. 5 to 8. The wavelength selection
beam splitters 33 and 34 having the wavelength
selection characteristic reflect the fluorescence to
thereby cut excitation lights λ_1 and λ_2 considerably.

On the other hand, positions 2201, 2202, ...,
15 22M on the sample are irradiated with the multi-spot
excitation light having the wavelength of 532 nm (λ_2)
different from the wavelength λ_1 . The sample absorbs
the multi-spot excitation light and when fluorescence
material ML2 emitting fluorescence is present, fluores-
20 cence having the wavelength λ_2' slightly longer than
the above wavelength is produced. The emitted fluores-
cence also shown in Fig. 4 passes through the objective
lens 3 having the large numerical aperture (NA) and
advances as shown by broken line with double arrows.
25 The fluorescence then transmits the wavelength
selection beam splitter 32 and is reflected by wave-
length selection beam splitters 31. This reflected
fluorescence transmits the wavelength selection beam

10053976.011802

splitter 34 (or 34') to enter the wavelength separation
fluorescence detection system 100. When the fluores-
cence is reflected by the wavelength selection beam
splitter 31 and transmits the wavelength selection beam
5 splitter 34 or 34', the excitation lights λ_1 and λ_2 are
cut considerably.

The relative position of the multi-spot
excitation lights having a plurality of wavelengths and
the sample is scanned once and a plurality of fluores-
10 cent marks or information of the fluorescence material
detected by the wavelength separation detection system
of Fig. 1 is stored once. When desired inspection
specifications such as, for example, a coordinate range
of an inspection area, information relative to an
15 object to be inspected and output conditions are
inputted by an input terminal 13 of Fig. 1, detection
is made in accordance with the conditions and a desired
output of detection results obtained is displayed on a
display monitor 12, for example. Further, the results
20 can be transmitted to a distant place through a line
14. Inputting of conditions of the above apparatus,
control of the excitation light, the stage and detec-
tion of a photo-multiplier, processing of the result,
outputting of the result and the like are controlled by
25 the control unit 1 of Fig. 1.

Fig. 5 shows an embodiment of the wavelength
separation fluorescence detection system 100. Numeral
1001 denotes a relay lens or a focusing lens. The

10053976-011802

fluorescence components emitted from different fluorescence materials ML1 and ML2 pass through the lens 1001 and are then separated into respective light fluxes at positions of interference filters 1211 and 1221. This separation is realized by shifting a position on the sample irradiated with both the excitation lights. Further, the separation is also realized by adjustment of positions or angles of the wavelength selection beam splitters 33 and 34. The separated fluorescence components λ_1 and λ_2 pass through the interference filters 1211 and 1221 to thereby reduce the excitation light component to an extremely small amount and remove noise in detection of fluorescence.

The fluorescence components λ_1' and λ_2' passing through the interference filters are reflected by wavelength selection mirrors 1212 and 1222 (or high-reflecting mirrors 1212' and 1222' of broadband) and enter a multi-channel photo-multiplier 101 shown in Fig. 6. Since the wavelength selection mirrors 1212 and 1222 transmit the excitation light component and reflect the fluorescence, the excitation light component is reduced to an extremely small amount equal to almost 0 after the reflection of the fluorescence. Since the wavelength selection mirrors 1212 and 1222 have respective reflecting planes which are not formed on one plane as shown in Fig. 5 and are inclined at an angle, both the fluorescence components reflected by the wavelength selection mirrors are focused on

10053976-011802

pinholes of a pinhole array or slits formed in a line at the front of the multi-channel photo-multiplier 101 as shown in Fig. 6.

Fig. 7 shows an embodiment showing a detection method effective for the case where the multi-channel photo-multiplier 101 is long in a direction perpendicular to the array direction. In this case, the mirror plane of the wavelength selection mirrors 1212 and 1222 of Fig. 5 is disposed on one plane. Further, when the excitation light can be cut sufficiently until this mirror is reached, a single usual mirror may be used.

In the embodiment, since a plurality of fluorescence components are detected by the single photo-multiplier (multi-channel photo-multiplier), the excitation light is turned on and off at different time zones. Semiconductor lasers having a wavelength of 635 nm and constituting the excitation light source are turned on and off by turning on and off a signal for a power supply for driving the lasers. On the other hand, a beam of the YAG laser in the plurality-of-wavelength excitation system 22 is turned on and off by means of an acousto-optic modulator (AO modulator) not shown.

Intensities I21 and I22 of the excitation lights having the wavelengths of 635 nm and 532 nm, respectively, have time zones different from each other as shown by (A1) and (B1) of Fig. 10 and in which the

20250326 011802

excitation lights are turned on and off. The turning on time of the excitation lights is several tens μ s to several hundreds μ s. In almost all fluorescence materials, since a delay time in emission of fluorescence after excitation is several ns to several hundreds ns, fluorescence is emitted in almost concurrence with an irradiation time of the excitation light. Accordingly, respective fluorescence components are detected in synchronism with turning on and off of the excitation light as shown by (A2) and (B2) of Fig. 10. (C) of Fig. 10 shows a relation of a movement amount or positional information S_t of the stage for holding the sample in synchronism with turning on and off of the excitation light and detecting fluorescence at different positions and time. That is, movement is made by Δx during a time Δt for detecting fluorescence components at 64 spots in response to turning on and off of the two excitation lights. This movement distance can be made approximately equal to a diameter d of the spot to thereby detect fluorescence in a desired area by means of scanning and turning on and off.

Fig. 8 shows an embodiment according to the present invention in which irradiation with excitation lights having a plurality of wavelengths are made at the same time to detect fluorescence at the same time. In this case, DNA sample or fluorescence material sample is irradiated with two (or three or more)

10053976.011802

excitation lights concurrently at the same place thereof (a different place may be irradiated). When the same place is irradiated with the excitation lights having the plurality of wavelengths, positions and
5 inclinations of the wavelength selection beam splitters 31 and 34 are previously adjusted. With such adjustment, fluorescence emitted by the excitation light having the wavelength of 635 nm, for example, can pass through the interference filter 1211 of Fig. 8 and
10 fluorescence emitted by the excitation light having the wavelength of 532 nm can pass through the interference filter 1221 of Fig. 8. Consequently, the respective fluorescence components can be reflected by the mirrors 1212' and 1222' and enter different multi-channel
15 photo-multipliers 1011 and 1012 to thereby detect the fluorescence components separately at the same time.

Fig. 9 is a diagram showing an embodiment according to the present invention in which three kinds of excitation lights are used by way of example.
20 Numeral 210 denotes a multi-spot excitation light system using a semiconductor laser having a wavelength of 635 nm (λ_{11}), 220 a multi-spot excitation light system using a wavelength of 590 to 600 nm (λ_{12}), and
230 a multi-spot excitation light system using a laser
25 having a wavelength of 532 nm (λ_{13}). A method of converting lights emitted from the respective light sources into multi-spots will be described using the multi-spot excitation light system 210.

20253976.011802

10053976-011302

The beam emitted from the light source is shaped to have a desired beam diameter and spread angle by means of a beam shaping unit not shown in drawings and is converted to have a desired polarization state by a polarizer 212. The polarized beam passes through beam splitters 213, 214 and 215. The beam splitters are each composed of an isosceles right triangle and a parallelogram with apical angles of 45 degrees having a hypotenuse stuck on a hypotenuse of the isosceles right angle and the stuck surface or side constitutes a beam splitting surface. Further, when the distance between the stuck side of the parallelogram and its opposite side of the beam splitter 213 is d , the corresponding distances of the beam splitters 214 and 215 are $2d$ and $4d$, respectively. Consequently, the beam passing through the beam splitter 215 is increased to 8 beams and the space between the beams is equal to $\sqrt{2}d$. The 8 beams form multiple spots having the desired beam diameter on 21A to 21B lines 2100 by means of convex lenses array 2161 to 2168.

Numerals 220 and 230 of Fig. 9 also represent multi-spot excitation optical systems having such structure. A wavelength selection beam splitter 202 transmits excitation light having a wavelength λ_{12} and reflects excitation light having a wavelength λ_{13} . A wavelength selection beam splitter 201 transmits excitation lights having wavelengths λ_{12} and λ_{13} and reflects excitation light having a wavelength λ_{11} .

10053976-01802

The multiple beams having 8 beams of the excitation lights having three wavelengths are increased to 32 beams equal to four times by means of beam splitters 203 and 205 made of, for example, calcite of birefringent material and wave plates 204 and 206 and form multiple spots by a desired beam system on straight lines 2200 and 2300 on surfaces 21A and 21B. The sample 5 is irradiated with the multiple spots of the three excitation lights formed on the straight lines 2100, 2200 and 2300 in this space at a desired spot diameter by means of the tubular lens 24 and the objective lens 3 described in Fig. 1.

Fig. 11 shows an embodiment according to the present invention in which there are two excitation wavelengths. In order to set a dynamic range for detection of fluorescence largely, it is necessary to change the intensity of excitation light to detect fluorescence. That is, when fluorescence having the number of molecules of fluorescence per unit area to be detected equal to an extremely small value to a large value such as, for example, one molecule/ μm^2 to 10 thousands molecules/ μm^2 is to be detected, the detection resolution (detection range/noise level) of a high-sensitivity detector such as a photo-multiplier is exceeded. Accordingly, this problem is solved by the method shown in Fig. 11. (A1) and (B1) of Fig. 11 have the abscissa representing time and the ordinate representing the intensities on the sample of

excitation lights having excitation wavelengths λ_1 and λ_2 , respectively. In the embodiment, detection using the excitation lights λ_1 and λ_2 is made in the time series manner, while the following description can be

5 similarly applied to the case where the detection is made at the same time. During time t_0 to t_2 , excitation is made by the excitation light λ_1 to detect fluorescence. During time t_0 to t_1 , excitation is made by strong light I1A as the intensity I21' of excitation

10 light and during time t_1 to t_2 , excitation is made by weak light I1B as the intensity I21' of excitation light. As shown by (A2) and (B2) of Fig. 11, fluorescence is detected by the excitation light intensities I1A and I1B in the zones t_0 to t_1 and t_1 to t_2 , respectively.

15

Similarly, during time t_2 to t_4 , as shown by (B1) of Fig. 11, the excitation light intensity I22' for the excitation light of λ_2 is set to I2A during time t_2 to t_3 and to I2B during time t_3 to t_4 . As

20 shown by (B2) of Fig. 11, respective fluorescence intensities are detected in respective time zones.

Fig. 12 shows a detection method of a signal of a photo-multiplier which can be applied to the case where the number of fluorescence molecules is very

25 small to the case where it is large. When the number of fluorescence molecules is small, a photon-counting method is applied. The photon-counting method is effective for the case where light is weakened and the

2025053976.01.1802

number of photon pulses is about several counts to
several hundreds or several thousands counts within a
detection time. Conversely, when fluorescence is
increased too much, photon-pulses are frequently
5 detected within a time width of a pulse signal and the
pulses cannot be counted exactly. In such case, as
described with reference to Fig. 11, by reducing the
fluorescence light intensity extraordinarily to
irradiate fluorescence with it, overlap of photon-
10 pulses can be reduced and fluorescence can be detected.

(A1) of Fig. 12 is similar to (A1) of Fig. 11
with the exception that the time axis of the abscissa
is enlarged. In the embodiment, three kinds of
fluorescence molecules are detected at the same time
15 while scanning the sample and the excitation light
relatively. That is, the sample is irradiated with two
or three kinds of excitation lights at the same time as
shown by (A1) of Fig. 12. Relative values of the
respective excitation lights are I1A, I2A and (I3A) and
20 I1B, I2B and (I1B) having extraordinarily weak inten-
sity. Counts of photon-pulses of fluorescence obtained
from three kinds of fluorescence materials L1, L2 and
L3 are shown by (C1), (C2) and (C3) of Fig. 12. The
fluorescence material L1 has approximately medium
25 intensity of fluorescence, L2 weak intensity, and L3
very strong intensity.

There are two pulse-counting methods includ-
ing a first method of counting a pulse having even a

10053976-011802

large pulse width as one pulse as shown in Fig. 12 and
a second method of measuring a time that a pulse signal
is high. In the first method, when the number of
pulses is increased, a count is reduced conversely, so
5 that the relation of the detection intensity and the
pulse count is not a single-valued function and the
count cannot be specified. On the contrary, in the
second method, even if the number of pulses is
increased, a count is not reduced and the relation of
10 the detection intensity and the pulse count is a
single-valued function, so that exact measurement can
be made by correcting the count. The second method can
make more exact counting, although when the width of
pulses is small, it is difficult to make exact counting
15 if the time constant of the counting circuit is not
made very small.

In the embodiment of Fig. 12, since irradiation with strong excitation light and weak excitation light is made to count photons in respective cases, the
20 counting can be made substantially without error even
in the first method that the single-valued function is
not obtained from the respective counts for the strong
and weak excitation lights. That is, in the case of
(C1) of Fig. 12, if a count obtained during time t_0 to
25 t_1 is not largely changed from a count obtained during
time t_1 to t_2 , it is understood that the latter value
is correct. Further, as shown by (C3) of Fig. 12, when
a count obtained during time t_0 to t_1 is small and a

10053976 " 011802

count obtained during time t_1 to t_2 is large, it is understood that the latter value is correct. On the other hand, as shown by (C2) of Fig. 12, when a count obtained during time t_1 to t_2 is almost 0, it is

5 understood that the count obtained during time t_0 to t_1 is correct.

Moreover, when a wider dynamic range is required, the intensity of the excitation light shown by (A1) of Figs. 11 and 12 is set to three stages
10 including a very strong excitation light, an approximately medium excitation light, and a very weak excitation light to thereby make it possible to attain detection in a wider dynamic range.

Fig. 13 shows an embodiment according to the
15 present invention in which only a fluorescence detection portion is shown. In the excitation light irradiation optical system, the wavelength selection beam splitters 201 and 202 are aligned so that three excitation lights 2100, 2200 and 2300 of Fig. 9 have
20 the same optical path. The sample 5 shown in Fig. 1 is irradiated with three multi-spot excitation lights 2100', 2200' and 2300' at the same place so that fluorescence components L1, L2 and L3 obtained are detected as follows. The fluorescence component L1 is
25 reflected by the wavelength selection beam splitters 32 and 34 of Fig. 1 and the fluorescence components L2 and L3 transmit the wavelength selection beam splitter 32, are reflected by the wavelength selection beam splitter

10053976 "011802"

31 and transmit the wavelength selection beam splitter
34 to thereby be led to the fluorescence detection
portion shown in Fig. 13. The fluorescence obtained
from the multi-spot excitation light on the sample is
5 focused on light receiving portion of the multi-channel
photo-multipliers 1011, 1012 and 1013 as a multi-spot
image by means of the objective lens 3 of Fig. 1 and
the tubular lens 1001 of Fig. 13. The fluorescence
components obtained from three fluorescent marks are
10 separated by means of wavelength separation beam
splitters 1231 and 1232 of Fig. 13. That is, the
fluorescence component L1 obtained by the excitation
light 2100' is reflected by the wavelength separation
beam splitter 1231, passes through an interference
15 filter 1241 which transmits only this fluorescence
component and then passes through an opening of a
multi-pinhole array 1010 to thereby be detected by the
multi-channel photo-multiplier 1011. Similarly, the
fluorescence components L2 and L3 pass through the
20 wavelength selection beam splitter 1231. The fluores-
cence component L2 transmits the wavelength selection
beam splitter 1232 and the fluorescence component L3 is
reflected by the wavelength selection beam splitter
1232. Both the fluorescence components then transmit
25 interference filters 1242 and 1243 and are detected by
the multi-channel photo-multipliers 1012 and 1013,
respectively.

In the embodiment shown in Fig. 13, three

10053976.011802

10053976-011802

wavelengths are used as the excitation light, while two wavelengths may be used as the excitation light to detect three fluorescence materials. That is, a semiconductor laser having a wavelength of 635 nm and a YAGSHG laser having a wavelength of 532 nm may be used as excitation light sources to detect Cy5 (R), Cy3.5 (R) and Cy3 (R) as fluorescence materials. In this case, since peak values of respective fluorescence spectra are in the vicinity of 670, 570 and 600 nm, the wavelength selection beam splitter 1231 is set to reflect the wavelength of 650 nm or more and transmit the wavelength smaller than 650 nm. Further, the wavelength selection beam splitter 1232 is set to reflect the wavelength of 585 nm or more and transmit the wavelength smaller than 585 nm.

With the detection of fluorescence as described above, by relatively scanning the excitation light and the sample on a slide glass over a desired area of, for example, 20 x 40 mm of the slide glass once, concentration of a plurality of fluorescence materials can be detected as fluorescence intensities.

In the description of the embodiment, the multi-spot array is used as the excitation light by way of example, while even a single spot or excitation light in the form of elongate sheet extending in the direction perpendicular to the scanning direction may be used to attain high-speed detection similarly.

It will be further understood by those

skilled in the art that the foregoing description has
been made on embodiments of the invention and that
various changes and modifications may be made in the
invention without departing from the spirit of the
5 invention and scope of the appended claims.

10053976-011802